i3D Symposium

First Line of Defense: Protective Immunity at Barrier Surfaces

Paul Robeson Cultural Center
350 Martin Luther King, Jr. Blvd
Newark, NJ

September 13 and 14, 2016
Mucosal and skin surfaces provide the first line of defense against invasion by potential pathogens. Our understanding of immune function at these barrier surfaces has advanced dramatically in recent years. Elucidation of the role of specific resident and recruited immune cell populations and their interactions with commensal and pathogenic micro- and macrobiota is now recognized as a critical area of research with the potential of providing significant advances in the development of new treatments and therapies for a variety of infectious and inflammatory diseases. In this symposium, internationally renowned researchers on the cutting edge of this field will present their recent findings and perspectives in this exciting area of research.
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<td>Essex 231</td>
<td>Breakfast and Registration</td>
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**Session I**  
**Impact of Microbiota on Intestinal Immunity**  
Chair: Sylvia Christakos, PhD

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<td>Cathryn Nagler, PhD</td>
<td>Innate immune regulation of sensitization to dietary antigens by commensal bacteria</td>
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<td>Eric Pamer, MD</td>
<td>Microbiota-mediated defense against intestinal infection</td>
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<td>Daniel Littman, MD, PhD</td>
<td>Control of T cell responses by the microbiota</td>
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**Break**

**Session II**  
**Barrier Immunity to Helminthes**  
Chair: George Hasko, MD, PhD

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<td>Parasites at the Gate: Regulation and Immunity</td>
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<td>Mark Siracusa, PhD</td>
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**Lunch**

**Session III**  
**Innate Defense at Mucosal Surfaces**  
Chair: George Yap, PhD

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<td>Sarah Gaffen, PhD</td>
<td>Straight from the Mouse’s Mouth: IL-17 Signaling in the Oral Mucosa</td>
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<td>Akiko Iwasaki, PhD</td>
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<td>Amariliz Rivera, PhD</td>
<td>Bidirectional Innate licensing orchestrates antifungal immunity in the lung</td>
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**Break**

**Keynote Speaker, David Artis, PhD**  
Introduction: Patricia Fitzgerald-Bocarsly, PhD

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Keynote Lecture

Immune regulation at barrier surfaces

David Artis
Michael Kors Professor of Immunology
Director, Jill Roberts Institute for Research in Inflammatory Bowel Disease
Weill Cornell Medicine
Cornell University
Joan and Sanford I. Weill Department of Medicine
Department of Microbiology and Immunology, New York NY

Employing models of microbial colonization, pathogen infection, chronic inflammation and tissue repair, research in the Artis lab is examining how mammalian host genetics and signals derived from the environment and commensal microbial communities influence innate and adaptive immune cell responses. We are employing gnotobiotic mice to examine the influence of defined commensal microbial communities on intestinal and peripheral immune cell development, function and influence on tissue homeostasis. Our recent findings indicate that commensal microbes have a significant regulatory influence on lymphocyte, innate lymphoid cell and granulocyte function associated with susceptibility to multiple infectious, allergic and metabolic disease processes. It is hoped that the results of these studies will advance understanding of the pathophysiology of multiple chronic inflammatory diseases, including asthma, allergy, inflammatory bowel disease and obesity, and provide a framework to test new therapeutic pathways to prevent and treat these diseases.
Molecular determinants of innate lymphocyte development and function

Aimee Beaulieu
Assistant Professor and Chancellor Scholar
Department of Microbiology, Biochemistry and Molecular Genetics, Center for Immunity and Inflammation, Rutgers New Jersey Medical School, Newark NJ

NK cells and ILCs are two important classes of innate lymphocytes with essential roles in early immune responses. Classical NK cells provide systemic protection against viruses and cancer, whereas ILCs tend to localize at barrier organs (e.g. the gut, skin, and lungs), where they defend against a broad range of pathogens, including viruses, helminths, and gut microbes. Current understanding of the pathways that control innate lymphocyte development and effector function is limited at best. Here we discuss our recent discovery of two novel and divergent molecular pathways - one involving the Inhibitor of Apoptosis (IAP) protein Birc5 and the other the BTB-ZF transcription factor Zbtb32 - that function as key regulators of cell cycle progression in developing and activated innate lymphocytes.
Intraepithelial lymphocytes (IEL) expressing the γδ T cell receptor are important regulators of the mucosal microenvironment in pathogen invasion and immune-mediated diseases, including inflammatory bowel disease and celiac disease. Located between adjacent intestinal epithelial cells, γδ IELs are ideally positioned to function as a first line of defense at the mucosal barrier. We have recently demonstrated that γδ IEL migration and active surveillance of the villous epithelium is essential to prevent the initial invasion of enteric pathogens. Within minutes following exposure to invasive Salmonella typhimurium, the frequency of γδ IEL localization into the epithelium is greatly enhanced. Further, the dwell time of γδ IELs in close contact with bacteria-infected epithelial cells is prolonged, indicating that γδ IELs may be able either to “sense” the presence of microbes or detect infected epithelial cells. To determine the mechanism by which gd T cells are recruited into the epithelium, we assessed gd IEL migration in response to IL-15, which is critical for IEL maintenance and contributes to host defense against infection of intracellular bacteria. We found that IL-15 increased gd IEL migration into epithelial monolayers in a dose-dependent manner and promoted gd IEL chemokinesis in vitro. Further, live imaging of transgenic mice overexpressing IL-15, specifically in the intestinal epithelium or lamina propria, showed that compartmentalized IL-15 overexpression induces differential migration of gd T cells within the intestinal mucosa. Taken together, our data indicate that IL-15 functions as a chemoattractant to promote gd IEL migration and surveillance of the villous epithelium.
The IL-17 family of cytokines are essential for immunity to fungal organisms, but also promote pathogenicity in various autoimmune disorders. In particular, humans and mice lacking signaling by the IL-17 receptor are prone to chronic mucosal infections with the commensal fungus Candida albicans. Mechanisms of IL-17R-dependent immunity in the context of Candida infections will be discussed.
The intestinal epithelia, enteric microbiota, and immune cells maintain a healthy homeostasis. Genetic and environmental factors that alter this homeostasis may underlie the pathogenesis of inflammatory bowel disease (IBD). Abnormal Paneth cell lysozyme expression or aberrant distribution of this protein in Paneth cells have been reported in IBD patients and mouse models containing IBD-associated mutations in ATG16L, NOD2, and LRRK2, respectively. Lysozyme is a β-glycosidase that specifically hydrolyze bacterial peptidoglycan, producing precursors of microbial agonists for multiple Pattern Recognition Receptors. The intestinal Paneth cells are the major sources of this enzyme in the small intestinal epithelia, however nothing is known about what exactly Paneth cell lysozymes physiologically do and whether lysozyme defects are indeed causal to some disease aspects seen in IBD pathogenesis. To directly address these questions, we derived novel Lyz1 knockout (Lyz1KO) mice that are deficient in Paneth cell specific lysozyme. Preliminary data showed that loss of Lyz1 caused enhanced transepithelial localization of bacteria and an altered microbiota profile. These microbial changes are associated with a reduced intestinal mucous layer, increased recruitment of lamina propria immune cells, and elevated levels of certain cytokines. These data may improve our understanding of complicated interactions between the mucosa and microbiota, and open intriguing questions about the delicate crosstalks between the mucosa and microbiota.
Macrophage-mediated protection in helminth infection

William C. Gause
Senior Associate Dean for Research
Director Center for Immunity and Inflammation
Professor, Department of Medicine
Rutgers New Jersey Medical School, Newark NJ

We have investigated the role of macrophages in mediating protective immunity during helminth infection. Our studies indicate that they mitigate tissue injury during helminth migration and can also promote resistance leading to decreased pathogen burden. Adoptive transfer of lung macrophages from mice inoculated with the nematode parasite, Nippostrongylus brasiliensis, mediates accelerated resistance in naïve recipients indicating that primed macrophages are sufficient to mediate effective memory responses. Lung macrophages from N. brasiliensis-primed mice but not from LPS inoculated mice rapidly adhere to parasites in vitro and directly mediate parasite killing through arginase-dependent mechanisms. Development of anti-helminth macrophages requires IL-4R but not IL-10 signaling and neutrophils are essential in their development. Analyses of macrophage subsets reveals that alveolar macrophages are particularly effective at parasite killing. Arginine supplementation in the in vitro model and in vivo effectively blocks parasite killing indicating that localized arginine depletion is an important mechanism. These studies thus demonstrate an important role for lung macrophages in mediating resistance and indicate a potential mechanism contributing to this effector function.
First Line of Intestinal Defense: Mucus and Mucins

Gunnar C. Hansson
Professor, Mucin Biology Groups, Department of Medical Biochemistry
University of Gothenburg, Sweden

The epithelial cell surfaces of the small and large intestine are protected by mucus, which serves as a first line of innate defense produced by surface goblet cells. The mucus is composed of many different molecules where the MUC2 mucins form the basic skeleton. The colon mucus is made up of two layers, an inner attached mucus that is normally not allowing bacteria to enter and an outer non-attached and expanded mucus layer that is the habitat of the commensal bacteria (Johansson et al., 2008). The inner mucus layer is however not static and largely affected by the bacteria (Jakobsson et al., 2015). The inner mucus was with one bacterial composition considerably more penetrable to bacteria and beads the size of bacteria. This abnormal property was transferred by the bacteria as was the phenotype with impenetrable inner mucus.

The small intestine on the other hand has only one type of mucus that is normally easily removed and penetrable to bacteria (Ermund et al., 2013). However, the bacteria are still held at a distance due to the numerous antibacterial compounds produced in this part of the intestine.

The MUC2 mucin skeleton forms a net-like polymer that is staggered on top of each other. We have recently discovered that this skeleton is enforced by isopeptide bonds formed between the amino acids Lys and Gln and catalyzed by the transglutaminase 2 (TGM2) (Recktenwald and Hansson, 2016). These stabilizing bonds are both formed when stored in the goblet cell granulae and after secretion and can be both intra- and intermolecular. This observation suggest common mechanisms between skin and intestinal protection.
Many body surface epithelia harbour organ-specific gd T cell compartments that enhance tissue integrity and increase resistance to carcinogens. How such compartments are established, maintained, and regulated is not well understood. In this regard, we showed that thymic epithelial cells and suprabasal keratinocytes uniquely express Skint1, a Butyrophilin-like (Btnl)/PDL-1-like gene that shapes the murine T cell receptor (TCR)-Vg5+ dendritic epidermal T cell (DETC) compartment. However, because neither Skint1 nor Vg5+ DETC is conserved, a general mechanism by which epithelia might shape local T cell compartments is elucidated. Here, tissue-specific expression of Btnl1 by murine enterocytes is shown to shape the signature intestinal TCR-Vg7+ compartment. Uninfluenced by microbial or food antigens, this process evokes Major Histocompatibility Complex (MHC)-mediated selection of TCRab+ repertoires. Indeed, Btnl1 together with Btnl6 provokes TCR-dependent responses specifically of intestinal Vg7+ cells. Moreover, human gut epithelial cells specifically express BTN3L and BTN3L8 that provoke selective TCR-dependent responses of signature intestinal Vg4+ cells. Hence, a conserved mechanism emerges by which organ-specific expression of BTN3L/Btnl genes permits epithelia to shape their local T cell compartments. Thus, the regulation of BTN3L/Btnl expression may regulate the immunogenicity of tissues in relation to infection, allergy, and cancer.
Antiviral defense at mucosal surfaces

Akiko Iwasaki
Professor of Immunobiology and of Molecular, Cellular, and Developmental Biology
Professor of Molecular Cellular and Developmental Biology
Howard Hughes Investigator
Yale Medical School, New Haven CT

Akiko Iwasaki’s research focuses on how the host recognizes and eliminates viruses that enter through mucosal surfaces. Her laboratory studies how viruses are detected by innate sensors and how that information is used to generate protective innate and adaptive immunity. Her systems include herpes simplex viruses in the genital tract, rhinoviruses and influenza infection in the respiratory tract. Her ultimate goal is to utilize the knowledge in the rational design of effective vaccines or microbicides for the prevention of transmission of viral pathogens.
The skin, like other barrier tissues, is exposed to numerous potentially pathogenic micro-organisms and houses a diverse array immune cell types. In particular, there are at least 3 subsets of dendritic cells (DC) found in the skin. In order to determine the relative importance of these DC subsets, we developed a skin infection model using *C. albicans* in which antigen-specific responses could be monitored in mice lacking individual DC subsets. We found that epidermal Langerhans cells (LC) were required for the development of Th17 cells while dermal CD103+ DC were required for the development of Th1 and the expansion of CD8 cells. Th17 cells provided protection against subsequent skin infections while Th1 provided protection against subsequent systemic infection. Dermal CD301b+ DC were neither necessary nor sufficient for the development of Th1 or Th17 due to the unavailability of Dectin-1 ligands on filamentous forms of *C. albicans* that invade the dermis. In contrast to the development of adaptive T cell responses, LC and CD103+ dDC were not required for early, innate response to *C. albicans*. Rather, dermal CD301b+ dDC were required for efficient production of IL-17 from skin-resident TCR γδ cells early during *C. albicans* infection through a mechanism that required DC interaction with pain sensing nociceptors and the release of IL-23. Thus, individual DC subsets drive distinct immune responses during both innate and adaptive responses and coordinate host defense to *C. albicans*. 
Distinct populations of mononuclear phagocytes have different functional roles in maintaining immunological homeostasis in the intestine. We and others have identified 3 populations of colon dendritic cells with unique abilities to drive T cell differentiation, as well as two populations of macrophages in the colon that express MHCII, F4/80, and high or low levels of CD11c. These macrophage populations have unique gene expression profiles, but also broadly shared expression of a spectrum of macrophage genes indicating further functional complexity within these populations. Both macrophage populations produce high levels of IL-10 and IL-1R-antagonist, but low levels of TNFa, IL-1b, IL-12, and IL-23 either constitutively or when activated with LPS or other TLR ligands in vitro indicating their anti-inflammatory functions. In addition, in recent studies, we found dramatically high mRNA levels and low protein expression for a select set of pro-inflammatory cytokines including TNFα and IL-6, as well as for the inflammasome proteins pro-IL-1b and NLRP3 in intestinal macrophages in comparison with bone marrow-derived and peritoneal macrophages. In contrast high mRNA but low protein production was not found for IL-10. Furthermore, activation of intestinal macrophages with TLR agonists resulted in up-regulation of mRNA for NLRP3 and IL-1b, but no increase in protein expression indicating a selective regulation of inflammatory genes at a post-transcriptional level. Furthermore, during experimental intestinal inflammation inflammatory monocytes and macrophages (CD64+F4/80+ cells) expressed high levels of both mRNA and protein for TNFα, IL-1b, and NLRP3 and produced high levels of IL-1b on activation of the NLRP3 and NLRC4 inflammasome indicating that microenvironmental signals during steady-state but not inflammatory conditions in the intestine control the expression of key inflammatory cytokines. Finally, blocking proteasome activity, as well as IL-10 signaling each resulted in enhanced production of NLRP3 and IL1b protein expression in intestinal macrophages from non-inflamed mice, suggesting novel mechanisms of post-transcriptional control of inflammasome activation and pro-inflammatory cytokine production by intestinal macrophages.
The skin is the largest organ of our body and plays a crucial role in protecting the host. While skin appears to be a mere covering of our body, it is rather a well-organized, complex organ, both at a cellular and molecular level which provide multiple functions for the host. Dr. Kim will discuss skin immunity and the sophisticated protective mechanisms that keep us healthy and homeostasis. She will also highlight a few common skin diseases to discuss what happens when the same mechanism goes awry and leads to inflammation, injury and disease state.
The vertebrate intestinal tract is colonized by hundreds of species of bacteria that outnumber the total cells in the host, yet must be compartmentalized and tolerated to prevent invasive growth and harmful inflammatory responses. A key function of commensal microbes is to contribute to the adaptive immune repertoire and to diverse lymphocyte effector functions. T cell responses against non-invasive commensals, as exemplified by responses elicited by the segmented filamentous bacteria (SFB) and Helicobacter hepaticus, contribute to shaping the repertoire of effector/memory and regulatory T cells. SFB adhere to the epithelium in the terminal ileum of mice and induce differentiation of Th17 cells that normally protect the mucosal barrier, but contribute to autoimmune disease in susceptible mice. H. hepaticus colonizes the large intestine and induces Treg cells in wild type mice and inflammatory Th17 cells in IL-10-deficient mice. How T cells elicited by commensal bacteria can influence autoimmunity is a central question that remains unsolved. Requirements for induction of Treg versus different types of Th17 cells will be discussed, as will be the potential role of serum amyloid A proteins in inflammation. These studies in mice are not only relevant for human autoimmune diseases, many of which have Th17 cell involvement, but may also provide insights into how commensal microbe-specific T cell responses could be harnessed for controlling pathogenic infections and cancer.
Inflammation plays vital roles in protective responses against pathogens and tissue repair, however, improper resolution of inflammatory networks is centrally involved in the pathogenesis of many acute and chronic diseases. Extensive advances have been made in recent years to define the inflammatory processes that are required for pathogen clearance, however, in comparison, less is known about the regulation of inflammation in sterile settings. Over the past decade non-communicable chronic diseases that are potentiated by sterile inflammation have replaced infectious diseases as the major threat to global human health. Thus, improved understanding of the sterile inflammatory process has emerged as one of the most important areas of biomedical investigation during our time. Our studies describe the cellular events and molecular signaling pathways regulated by NLRs and IL-1 that govern sterile inflammation in autoinflammatory disease.
Hair follicle-mediated regulation of skin immunity

Keisuke (Chris) Nagao  
NIH Stadtman Investigator  
Dermatology Branch  
Center for Cancer Research  
National Cancer Institute, Bethesda MD

This skin is the outermost barrier and an active site for immune responses. It is inhabited by a variety of resident leukocytes. However, the mechanisms that regulate recruitment and persistence of these cells at baseline and in inflammatory or malignant skin diseases remain incompletely characterized. We have found in the recent years that hair follicles are immunologically active structures capable of recruiting dendritic cells to sites of minor trauma via the production of chemokines. We further explored another immunological aspect of the hair follicles that govern resident memory T cell homeostasis in the steady state and lymphoma.
Innate immune regulation of sensitization to dietary antigens by commensal bacteria

Cathryn Nagler  
*Bunning Food Allergy Professor*  
Department of Pathology, Department of Medicine, Committee on Immunology, Committee on Molecular Metabolism and Nutrition, Molecular Pathology & Molecular Medicine (MPMM)  
The University of Chicago, Chicago IL

Immunoregulatory responses induced by commensal bacteria are critical to preventing intestinal inflammation. Whether the intestinal microbiota also plays a role in regulating non-responsiveness to the other major luminal constituent - food - has been poorly understood. Murine models developed in our laboratory demonstrate that sensitization to a food allergen is enhanced in mice that have been treated by neonatal antibiotic administration (Abx) or are devoid of commensal microbes (germ free). By selectively colonizing germ free mice we showed that the allergy-protective capacity is contained within the Clostridia, a class of anaerobic spore-forming Firmicutes that resides in close proximity to the intestinal epithelium. Reintroduction of a Clostridia-containing microbiota to Abx-treated mice blocks sensitization to a food allergen. Microarray analysis of intestinal epithelial cells isolated from gnotobiotic mice identified a novel innate mechanism by which Clostridia protect against sensitization to dietary antigens. Clostridia colonization induces the production of the barrier protective cytokine IL-22 by both innate lymphoid cells and T cells in the intestinal lamina propria. IL-22-mediated effector functions, including the production of mucus and anti-microbial peptides, collectively contribute to protection against sensitization by reducing the access of dietary antigen to the systemic circulation. Our mouse model work is supported by translational studies comparing the fecal microbiota of healthy infants to that of infants with cow’s milk allergy (CMA). We find that the CMA infant microbiome has the diverse community structure typical of adults. Treatment of CMA infants with a tolerance inducing formula supplemented with the probiotic *Lactobacillus rhamnosus* GG (LGG) is associated with changes in microbial community structure that include the expansion of butyrate-producing Clostridia and significantly higher levels of butyrate detectable in feces. Butyrate, but not other short chain fatty acids, regulates epithelial barrier function in our mouse model. Commensal bacteria produce butyrate by fermentation of insoluble dietary fiber and we also find that mice weaned onto high fiber diets exhibit reduced intestinal permeability to food antigens. Further elucidation of the mechanisms by which innate immune signals from commensal bacteria and their metabolites regulate the intestinal epithelial barrier will inform the development of novel microbiome modulating approaches to prevent or treat sensitization to food.
Infections caused by antibiotic-resistant bacteria generally begin with colonization of mucosal surfaces, in particular the intestinal epithelium. The intestinal microbiota provides resistance to infection with highly antibiotic-resistant bacteria, including Vancomycin Resistant Enterococcus (VRE) and *Clostridium difficile*, the major cause of hospitalization-associated diarrhea. Metagenomic sequencing of the murine and human microbiota following treatment with different antibiotics is beginning to identify bacterial taxa that are associated with resistance to VRE and *C. difficile* infection. We demonstrate that reintroduction of a diverse intestinal microbiota to densely VRE colonized mice eliminates VRE from the intestinal tract. While oxygen-tolerant members of the microbiota are ineffective at eliminating VRE, administration of obligate anaerobic commensal bacteria to mice results in a billion-fold reduction in the density of intestinal VRE colonization. Many antibiotics destroy intestinal microbial communities and disable the native microbiota’s ability to inhibit *C. difficile* growth and toxin production. Which intestinal bacteria provide resistance to *C. difficile* infection and their in vivo inhibitory mechanisms remains unclear. By treating mice with different antibiotics that result in distinct microbiota changes and lead to varied susceptibility to *C. difficile*, we correlated loss of specific bacterial taxa with development of infection. Mathematical modeling augmented by microbiota analyses of hospitalized patients identified resistance-associated bacteria common to mice and humans. Using these platforms, we determined that *Clostridium scindens*, a bile acid 7-dehydroxylating intestinal bacterium, is associated with resistance to *C. difficile* infection and, upon administration, enhances resistance in a secondary bile acid-dependent fashion. Using a workflow involving mouse models, clinical studies, metagenomic analyses and mathematical modeling, we identified a probiotic candidate that corrects a clinically relevant microbiome deficiency. Our studies indicate that obligate anaerobic bacteria enable clearance of intestinal VRE colonization and may provide novel approaches to prevent the spread of highly antibiotic-resistant bacteria.
Bidirectional Innate licensing orchestrates antifungal immunity in the lung

Amariliz Rivera
Assistant Professor, Department of Pediatrics and the Center for Immunity and Inflammation
Rutgers New Jersey Medical School, Newark NJ

The number of patients susceptible to invasive fungal infections across the world continues to rise at an alarming pace yet current antifungal drugs are often inadequate. Immune-based interventions hold the promise of significantly improving patient outcomes however our understanding of relevant targets for antifungal defense is limited. Innate cells are important direct effectors of fungal pathogen eradication and low numbers of neutrophils or monocytes results in enhanced susceptibility to invasive fungal infections. It has thus been assumed that enhanced susceptibility to fungal infections in patients with low innate cell counts is due only to the absence of effector cells. Recent findings from my lab indicate that monocytes and their derivative cells are direct antifungal effectors as well as regulators of neutrophil antifungal function. We find that in the absence of monocytes, neutrophils are less capable of inactivating fungal cells. Similarly, we find that in the absence of neutrophils, monocytes are less capable of eliminating fungal cells. Thus, these two effector cell populations operate so that their sum is much greater than their parts. Our studies suggest that monocytes and neutrophils engage in bidirectional innate cell licensing for optimal fungal pathogen eradication. By deciphering the factors that mediate this pathway we will identify novel immune-based therapeutic targets. Using a combination of methods to measure antifungal immunity and a discovery-based systems biology approach we have developed a platform to identify novel factors in antifungal immunity. The utility of this unbiased platform for the discovery of candidate factors that might control bidirectional innate cell licensing will be discussed.
The immune system has evolved to mount an effective defense against pathogens and to minimize deleterious immune-mediated inflammation caused by commensal microorganisms, immune responses against self and environmental antigens, and metabolic inflammatory disorders. Regulatory T (Treg) cell-mediated suppression serves as a vital mechanism of negative regulation of immune-mediated inflammation and features prominently in autoimmune and auto-inflammatory disorders, allergy, acute and chronic infections, cancer, and metabolic inflammation. The discovery that Foxp3 is the transcription factor that specifies the Treg cell lineage facilitated recent progress in understanding the biology of Treg cells. The cellular and molecular mechanisms in the differentiation and function of these cells will be discussed.
Pathogenesis and Transmission of Mycobacterium tuberculosis

Padmini Salgame
Professor, Department of Medicine
ICPH, Newark NJ

Despite inroads in the development of new diagnostics, vaccine candidates and drugs, tuberculosis (TB) continues to endanger global public health. A major gap in knowledge is an incomplete understanding of the transmission dynamics of Mycobacterium tuberculosis (Mtb). In a study of household contacts (HHC) of infectious TB cases conducted by us in Brazil, “index” cases were categorized into High (HT) and Low (LT) transmission groups based on the proportion of household contacts with a positive tuberculin skin test. This presentation will discuss our ongoing studies with the Mtb isolates derived from HT and LT groups in understanding the mechanistic basis for the divergence in their transmission phenotypes. Overall, our findings suggest that distinct early innate immune interactions between microbe and host lead to differential trajectory in intracellular growth pattern and lung pathology that underlie differences in transmission potential.
The limited binding footprint, orientation and high affinity of the NKT TCR for its ligand makes this interaction distinct from conventional TCR:MHC interactions. The relative rigidity of the NKT TCR CDR loops is also unique for a TCR. Molecular modeling suggested that a hydrophobic patch created upon TCR a/b pairing might play a role in maintaining the NKT TCR conformation. Disruption of this patch ablated recognition of CD1d, but did not interfere with MHCI or MHCII interactions. Partial ablation, while not compromising CD1d binding, did substantially alter NKT cell development, resulting in to the selective accumulation of adipose tissue-specific NKT cells. Therefore, we have uncovered a key component of the TCR that is essential for the development of a distinct sub-lineage of NKT cells.
Type 2 cytokine responses are necessary for the development of protective immunity to helminth parasites but also cause the detrimental inflammation associated with allergies and asthma. Recent studies have found that peripheral hematopoietic progenitor cell populations contribute to type 2 cytokine-mediated inflammation through their enhanced ability to develop into mast cells. Here we identify that carbonic anhydrase (Car) enzymes are upregulated in type 2-associated progenitor cells and demonstrate that Car enzyme inhibition was sufficient to prevent murine mast cell development, type 2 cytokine-mediated inflammation and protective immunity to Trichinella spiralis. Moreover, we show that Car enzyme inhibition was also sufficient to prevent intestinal mast cell responses in a murine model of food allergy-like disease. Finally, we performed translational studies and demonstrated that Car enzymes can be targeted with an FDA-approved inhibitor to prevent human mast cell development. Collectively these studies identify a previously unrecognized role for Car enzymes in regulating mast cell lineage commitment and suggest that FDA-approved Car enzyme inhibitors may possess additional off-label therapeutic potential that can be employed to treat mast cell-mediated inflammation.
The gut microbiota provides a first line of defense against Listeria monocytogenes

S Becattini, SG Kim, RA Carter, L Ling, IM Leiner, EG Pamer

Immunology Program, Memorial Sloan Kettering Cancer Center, New York, NY

Listeria monocytogenes is an important cause of food borne infections, particularly in patients with hematologic malignancies, causing septicemia and meningoencephalitis. Although most strains of L. monocytogenes are antibiotic sensitive, antibiotic treatment is frustratingly ineffective at curing highly immunocompromised patients, resulting in a mortality rate of approximately 30%. The gut microbiota, which consists of commensal bacterial populations that inhabit the intestine, normally confers protection against orally acquired pathogens by exerting colonization resistance. Changes in microbiota composition induced by disease, pharmacological or antibiotic therapies can lead to dysbiosis, thereby impairing commensal-mediated colonization resistance and predisposing to infection.

Our preliminary data show that bacterial species represented in a healthy microbiota can efficiently eliminate Listeria monocytogenes from the gut lumen, thus preventing the pathogen from translocating across the intestinal epithelium and spreading systemically.

Microbiota-mediated protection results particularly important in the context of congenic or acquired (chemotherapy-driven) immune deficiencies. Indeed, mice lacking T, B lymphocytes as well as innate lymphoid cells (Raggc mice), are highly susceptible to doses of oral Listeria that are non-lethal in WT mice, as a result of the absence of innate immune cells producing interferon gamma at early stages. Interestingly, Raggc mice are capable of surviving Listeria infection if challenged with low doses, that are likely to mimic the size of bacterial burden encountered by hospitalized patients. However, Raggc mice pre-treated with antibiotics experience uncontrolled intestinal expansion of Listeria and subsequent systemic spread, succumbing even to low inocula. This phenotype could be reproduced in mice treated with a combination of doxorubicin and cyclophosphamide, a cocktail often used in the treatment of cancer, which became highly susceptible to low doses of Listeria only when pre-treated with antibiotics. As antibiotics often accompany anti-cancer therapy in the clinics, we believe these findings could have a remarkable translational relevance.

We are in the process of identifying the specific taxa of commensal bacteria and molecular mechanisms that mediate protection against L. monocytogenes. Our data indicate that both genera Lactobacillus, in the small intestine, and Clostridium, in the large intestine, contribute to outcompete Listeria, thus lowering the bacterial burden and the chance of systemic spread. Germ-free mice reconstituted with a consortium of putative protective bacteria show improved resistance to infection than untreated GF mice, or GF mice reconstituted with microbiota of antibiotic-treated mice, making a strong case for gut commensals to be independent and crucial players in the host response against Listeria.
IL-4R signaling in B cells mediates control of IL-17A dependent emphysema triggered by N. brasiliensis infection

F Chen, W Wu, A Millman, M Palma, WC Gause
Department of Medicine, Rutgers New Jersey Medical School, Newark, NJ

The intestinal nematode parasite, Nippostrongylus brasiliensis (Nb), transiently resides in the lung and causes lung damage, which is then rapidly repaired. However, the repaired lung eventually develops emphysema and the mechanisms contributing to this chronic lung pathology remain poorly defined. Here we show that emphysema, as measured by mean linear intercept measurements (Lm) of alveolar spaces, develops as early as 7 days after Nb inoculation and is associated with increased gene expression of Il17a, neutrophil elastase (ELA2), and macrophage matrix metalloproteinase 12 (Mmp12) in the lung. Transfer of Nb-primed neutrophils or macrophages to naïve recipients results in emphysema, and these cells expressed high level of ELA and Mmp12, respectively. Neutralization of IL-17A using anti-IL-17A antibody curbed development of emphysema, and decreased influx of neutrophils in the lung and gene expression of Il17a. Parasite infection in B cell deficient Jh-/ mice results in a more severe emphysema when compared to wild-type mice or FcR¿/- mice, and is associated with higher numbers of neutrophils and macrophages, as well as increased expression of Il17a, ELA2 and Mmp12 in the lung. Transfer of B lymphocytes from wild-type mice or Il10/- mice, but not from Il4Ra/- mice, to recipient Jh/- mice can restore control of emphysema and downregulate IL-17A. B cells from lung tissue of Nb-primed wild type but not Il4Ra/- mice expressed high level of Relma. Taken together, these data suggest that IL-17A triggers infiltration of neutrophils and macrophages that contribute to chronic emphysema, which is controlled IL-4R dependent B regulatory cells expressing high levels of Relma.
H2-O (HLA-DO in humans) is a non-classical MHC II-like molecule that inhibits H2-M (HLA-DM), which catalyzes peptide loading onto MHCII. Its role in the immune response remains inconclusive. More importantly, the role of H2-O during Mycobacterium tuberculosis (Mtb) infection has not been explored. However, it was shown that H2-O deficient B cells preferentially occupy the germinal center due to a greater ability to present antigen-derived peptides by MHCII molecules and provide T-cell help. Several studies have found that Mtb interferes with antigen-presentation and delays the arrival of CD4 T cells to the lungs. We therefore hypothesized that H2-O deficient mice will exhibit enhanced host resistance against Mtb infection because of their superior ability to increase antigen presentation. We infected wild type and H2-O deficient mice with Mtb Erdman by the aerosol route. Interestingly, the H2-O deficient mice exhibited decreased bacterial burden in the lungs compared to WT mice during the course of infection. This decreased bacterial burden was observed as early as four weeks and remained consistently low throughout the 16 week infection period. Moreover, bacterial load of H2-O deficient mice in the spleen and draining lymph nodes were also decreased suggesting reduced extrapulmonary dissemination. Consistent with decreased bacterial burden, we observed a lower level of cellular recruitment in the lungs of H2-O deficient mice. Histopathological evaluation demonstrated decreased pulmonary inflammation with reduced cellular infiltration in H2-O deficient mice compared to WT mice. We plan to further examine the mechanistic basis for increased bacterial control in the H2-O KO mice. Together, these results indicate that blocking H2-O could be a novel means to enhance Mtb antigen presentation and host resistance to Mtb infection.
Plasmacytoid dendritic cells (pDCs) function as sentinels against viral infection in humans by producing abundant amounts of Type-I and Type-III interferons as well as other cytokines. Detection of viral nucleic acids remains the major initiating factor for interferon and cytokine production in pDCs. Although the Toll-like receptor-mediated endosomal pathway of nucleic acid detection has been studied and documented in great detail, detection of cytoplasmic DNA in human pDCs remains, for the most part, unexplored. Recently, a cytoplasmic pathway of DNA recognition and interferon expression has been discovered in the murine system and certain human cell lines. This pathway is mediated by the cytoplasmic protein cGAMP synthetase (cGAS), which initiates Type-I interferon production via a cyclic dinucleotide called 23cyclic GMP-AMP (cGAMP), which in turn, activates the endoplasmic reticulum resident protein Stimulator of Interferon Genes (STING) and leads to cytokine expression. Here, for the first time, we demonstrate the existence of components of the cGAS/STING pathway present in human pDCs. Our data indicate that both cGAS and STING are transcribed and translated in primary human pDCs. We have also confirmed the functionality of the pathway by inducing cytokine (IFN-α, IFN-γ, TNF-α, IL-6) production through stimulating pDCs by exogenous cGAMP. Our study suggests that this cGAS-STING-mediated cytokine response exists in parallel to the TLR9-mediated DNA recognition and immune activation in human pDCs.
Genotype-specific differences dictate disease outcome in Nippostrongylus brasiliensis and Mycobacterium tuberculosis coinfected mice

JM Dietzold, A Gopalakrishnan, WC Gause, P Salgame
Department of Medicine, Rutgers University-New Jersey Medical School

Previously, we reported that Nippostrongylus brasiliensis (Nb), an intestinal helminth, exacerbates Mycobacterium tuberculosis (Mtb) disease in coinfected BALB/c mice through the generation of alternatively activated (M2) macrophages. In order to gain further mechanistic insight, we determined whether host genetic factors contributed to disease exacerbation in coinfected animals. To investigate this possibility, coinfection experiments were performed in C57BL/6 mice. In contrast to the enhanced susceptibility observed in the BALB/c mice, coinfected C57BL/6 mice were able to control Mtb burden that was comparable to mice infected with Mtb alone, despite the induction of a Th2 response. A comparative gene expression analysis between Nb-infected BALB/c and C57BL/6 mice demonstrated that the lung milieu prior to Mtb infection is starkly different between the two genotypes. BALB/c mice exhibited a stronger Th2 response and a gene expression profile consistent with increased iron availability by the macrophages in the lung. Consistent with this, in vitro experiments we found that M1 Macs have an iron storage phenotype such that there is restricted access to iron by intracellular Mtb whereas in M2 Macs free iron is accessible to intracellular Mtb. Because Mtb requires iron for intracellular growth, these findings implicate dissimilarity in iron handling by M1 and M2 Macs as the operant mechanism leading to enhanced Mtb growth in M2 Macs in coinfected animals. Overall these data provide a better understanding of how helminth coinfections modulate TB disease severity and treatment outcome.
Neutrophils license antifungal monocyte responses via carbonic anhydrase 4 modulation

V Espinosa, O Dutta, E Henry, M Siracusa, A Rivera

Department of Pediatrics, Rutgers New Jersey Medical School
Newark, NJ

Neutropenia is a significant risk factor for life threatening invasive fungal infections (IFI). Neutrophils are well known as important innate cells that promote the direct eradication of fungal pathogens, but whether they mediate antifungal defense beyond their role as effectors is unclear. Here we demonstrate that a pulmonary infection with the clinically relevant fungal pathogen, Aspergillus fumigatus, induces the diversification of specialized antifungal neutrophils that are required for antifungal CCR2+ monocyte-derived dendritic cell (mo-DC) function. Selective depletion of neutrophils resulted in global transcriptional alterations of the antifungal CCR2+ monocyte response, limited mo-DC differentiation, and diminished conidialicidal activity. Impaired mo-DC antifungal activity in neutropenic mice was accompanied by significant up-regulation of carbonic anhydrase 4 (Car4) expression in CCR2+ monocyte precursors. Pharmacological inhibition of Car4 with the FDA-approved drug methazolamide (MZ) rescued the antifungal response of mo-DC and protected neutropenic mice from invasive aspergillosis. Thus, beyond their role as effectors, antifungal neutrophils facilitate antifungal mo-DC functions by regulating Car4 activity. Moreover, our data provide proof-of-principle evidence for the importance of carbonic anhydrases in shaping innate cell differentiation as well as for the off-label therapeutic benefit of carbonic anhydrase inhibitors to boost antifungal immunity in susceptible patient populations.
The mRNA-protein interactome coordinates post-transcriptional gene regulation in insect-form Trypanosoma brucei

MA Fisher, A Das, and V Bellofatto

Department of Microbiology, Biochemistry, and Molecular Genetics; Rutgers New Jersey Medical School, Newark, NJ

The vast majority of the genome of the harmful human parasite Trypanosoma brucei is constitutively transcribed. Post-transcriptional mechanisms are therefore primarily responsible for regulating dynamic gene expression patterns throughout the T. brucei life cycle. T. brucei must be metabolically nimble to survive its diverse insect vector and mammalian host environments. In the insect vector, survival likely requires a unique mRNA-protein interactome that coordinates post-transcriptional gene regulation. Briefly, we used zero-distance UV-crosslinking to covalently fasten proteins to RNAs in live T. brucei insect-form cells, stringent oligo d(T)-based isolations to enrich for poly(A)+ RNA-protein complexes, and mass spectrometry to identify mRNA-binding proteins (mRBPs). We detected 1,216 proteins that reproducibly interact with mRNAs. Experimentally-validated and predicted mRBPs and RBPs are highly represented in this T. brucei insect-form mRBPome, and 377/1,216 (31%) are also present in the T. brucei bloodstream-form mRBPome. Furthermore, there is significant overlap between yeast, worm, human, and T. brucei insect-form mRBPome orthologs, suggesting evolutionary conservation of a core mRBPome. Intriguingly, most metabolic enzymes involved in energy production in the T. brucei insect-form glycosome are part of the mRBPome. We will explore this glycosomal mRNA-protein interactome in detail, along with several other novel mRNA-protein interactions revealed by the T. brucei insect-form mRBPome. We will ultimately determine which of these proteins alter transcript stability or affect translation rates, and which poly(A)+ RNAs function as effectors of protein activities, control protein localization, or act as scaffolds for multi-protein complexes.
Poster: #8

Role of Toll-Like Receptor 2 in protection against the hypervirulent Mycobacterium tuberculosis strain HN878

Archana Gopalakrishnan, Jillian Dietzold and Padmini Salgame
Rutgers School of Biomedical Sciences, Newark, NJ

Previously we reported that Toll-Like receptor (TLR) 2 is dispensable for host protection against acute infection with Mycobacterium tuberculosis (Mt) Erdman, but essential for maintaining stable granulomatous response and bacterial burden during chronic infection. In this study we examined the requirement for TLR2 in host immunity against the hypervirulent clinical Mt strain HN878. We found that akin to infection with Mt Erdman, TLR2KO mice infected with Mt HN878 also showed significantly increased bacterial burden in the lungs compared to WT animals. However, this increase in bacterial load was seen as early as 4 weeks in the Mt-HN878-infected TLR2KO mice and remained significantly higher than that of the WT mice throughout the 10 week infection period. Histopathological evaluation demonstrated increased pulmonary inflammation with enhanced infiltration of lymphocytes and neutrophils in Mt HN878-infected TLR2KO mice compared to WT mice. Consistent with increased neutrophil accumulation, we observed an increase in the expression of CXCL-5, a neutrophil chemoattractant in the lungs of TLR2KO mice. Interestingly, IL-17 expression was also significantly upregulated in the lungs of the TLR2KO mice suggesting that IL-17 may also mediate the neutrophil accumulation. Furthermore, unlike with Mt Erdman infection, no apparent differences in regulatory T cell accumulation in the lung granulomas were observed between the WT and TLR2KO mice. In vivo blocking of neutrophil (anti-Ly6G) in TLR2KO reversed the phenotype in these mice. Bacterial burden in the lungs of these mice were decreased to levels similar to WT infected mice. Together, these findings reveal that TLR2 is necessary for host protection against a hypervirulent clinical strain and highlight that different Mt strains may utilize different immunoregulatory pathways to control immunopathology in the lung. Currently we are examining the mechanistic basis that leads to the differential induction of immunoregulatory pathways by Erdman and HN878 strains.
Dysregulation of the biogenesis of lipid droplets (LD), cytoplasmic organelles rich in neutral lipids, is linked to metabolic and infectious diseases. The relative lipid and protein composition of LD varies with cell type and/or the infectious agent implicated in their accumulation. In tuberculosis, LD formation in macrophages is a critical step in the development of foamy macrophages within the granuloma, which is the hallmark tuberculous lesion. Among mycobacteria-infected macrophages, characterization of lipid species has been performed only for macrophages infected with M. leprae: these cells show increment of free and esterified cholesterol and reduction of triglycerides (TAG). Since infected macrophages accumulate compositionally different LD in a pathogen-specific manner, we set out to characterize the lipid species found in LD induced in M. tuberculosis-infected macrophages.
Transcranial Stimulation Attenuates Local Inflammation In Arthritis

Joseph B., Ulloa L

Department of Surgery, New Jersey Medical School, Newark, NJ

B Joseph1*; GS Bassi2; DP Dias3; VR Santos3; M Franchin4; GB Menezes5; DG Reis4; J Talbot4; J Castania3; FD Vecchio3; L Resstel4; HC Salgado3; FQ Cunha4; T. Cunha4; NG Cairasco3; A Kanashiro4 and L Ulloa1, 6

Department of Surgery1 and Center of Immunology and Inflammation6, Rutgers - New Jersey Medical School; Departments of Physiology3 and Pharmacology4 and Immunology2, University of São Paulo, Ribeirão Preto, SP, Brazil; Department of Morphology5, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

Abstract shown at symposium
M. smegmatis vaccination reveals the mycobacterial ribosome as a potential CD4+ Tcell enhancing vaccine target

Steven Kennedy
Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York

Mycobacterium tuberculosis (Mtb) is an airborne pathogen that infects a third of the world's population. To date no vaccine has had major success in reducing the enormous global burden of disease from this infection. Critical to the control of Mtb is the ability of a vaccine to induce a strong CD4+ Tcell response. The currently available vaccine, M. bovis BCG, is of limited efficacy, potentially due to its retention of immune evasion mechanisms that actively inhibit the host presentation of critical antigens to CD4+ Tcells. Examining antigens that are hidden during a BCG immunization may lead to identification of novel vaccine targets. Our lab has previously identified an M. smegmatis based vaccine, IKEPLUS, which elicits a strong CD4+ Tcell response leading to significantly enhanced protection to Mtb over BCG vaccination. M. smegmatis is a non-pathogenic mycobacterium that has not retained the ability to disrupt antigen presentation to the host immune system. Examination of the CD4+ Tcell response to IKEPLUS and BCG has identified the mycobacterial ribosome as a major immunogenic target in the M. smegmatis based IKEPLUS, but not in BCG. This dichotomy in the immune response led us to further characterize the ribosome and evaluate its protein constituents as potential vaccine targets. The ribosome is composed of 57 individual proteins; of these 57 proteins, we have identified 23 as immunogenic, and identified minimal CD4+ Tcell epitopes for 16 of the 23 identified proteins. These findings have led us to begin development of a mycobacterial ribosome based vaccine to enhance the currently existing BCG regimen. This approach will allow us to complement what we believe is a critical flaw of BCG while retaining the widely used BCG vaccination regimen.
Poster: #12

Transcriptome analysis of Mycobacterium tuberculosis-specific CD4+ T cells identified by CD154 expression

S. Kunnath-Velayudhan, M. F. Goldberg, S. A. Porcelli
Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York

Characterization of antigen-specific CD4+ T cells is critical for understanding immune responses to infection and vaccination. Analysis of these cells at transcriptome level can inform us about the breadth of the immune response in an unbiased fashion but it has been technically challenging. Though intracellular cytokine staining can identify cells that produce cytokines upon stimulation with specific antigen, isolation of high quality RNA from fixed and permeabilized cells remains a challenge. Cytokine-capture assays allow isolation of live cells that produce cytokines but are laborious and have limited sensitivity. Identification of antigen-specific CD4+ T cells using multimers of MHC II molecules is also limited by the need for synthesis of peptide-MHC tetramer of interest and by heterogeneity of MHC II. Expression of CD154 upon stimulation with cognate antigen has been shown to identify antigen-specific CD4+ T cells from human peripheral blood mononuclear cells but application of this approach for transcriptomic analysis has not been pursued. Here we show that in mouse, expression of CD154 identifies adoptively transferred ovalbumin-specific CD4+ T cells or endogenous antigen-specific cells induced by Mycobacterium bovis BCG vaccination. In these systems, antigen-specific cytokine production was positively correlated with CD154 expression. We further show that high quality microarrays can be performed from RNA isolated from CD154 positive cells from M. bovis BCG-vaccinated mice. Principal component analysis of the microarray data showed that transcriptome of CD154 positive and negative population clustered separately indicating distinct transcriptome signatures for these cells. Gene set enrichment analysis showed that the transcriptome of CD154 positive cells are enriched for metabolic pathways of T-cell activation. Additional analysis of microarray data showed that the transcripts that code for signature cytokines of Th1, Th2, Th17 and Tfh but not regulatory T helper cells are enriched in the transcriptome of CD154 positive cells. Furthermore, we show that transcriptome analysis of CD154 positive CD4+ T cells can be used to compare immune signatures of different vaccine candidates. Overall, our results support the use of CD154 expression upon antigen stimulation as a reliable method to identify and perform transcriptome analysis of antigen-specific CD4+ T cells.
Plasmacytoid Dendritic Cells (pDCs) are potent producers of type-1 interferons (IFN) and are vital to a successful antiviral immune response. Recent studies have shown pDCs also play an important role in antifungal immunity. Here, we demonstrate the ability of human pDCs to uptake and kill fungal spores, and characterize expression of the C-type Lectin Receptor (CLR) Dectin-1, which we have identified as a likely receptor used to recognize fungal antigen. We also compare Dectin-1 expression between chronic HIV-infected patients and healthy controls, due to the observation that HIV patients experience chronic immune activation and pDC dysregulation that may compromise pDC antifungal capability. Aspergillus fumigatus spores were labeled with Fluorescent Aspergillus Reporter (FLARE), a tracking system that allows the user to track the presence and viability of spores using flow cytometry. FLARE-labeled Aspergillus spores were incubated with healthy peripheral blood mononuclear cells (PBMC). After 6 hours, pDCs were evaluated for acquisition of FLARE signal. To investigate pDC Dectin-1 expression, we used flow cytometry to measure Dectin-1 protein levels on the pDC cell surface at baseline and in response to HSV, HIVMN, and AT2-HIVMN stimulation. To evaluate Dectin-1 functionality, we stimulated pDCs with curdlan, a specific Dectin-1 agonist, and measured the upregulation of costimulatory markers, Dectin-1, and cytokine production. Finally, we compared pDCs of individuals with chronic HIV to those of healthy controls for the expression and regulation of Dectin-1 at baseline or after stimulation with curdlan, HSV, HIVMN, or AT2-HIVMN. Human pDCs picked up FLARE signal in a dose-dependent manner, which was inhibited by cold conditions. A very low percentage of cells that contained AF633 signal also contained DSRed signal, indicating decreased spore viability after uptake by pDCs. Dectin-1 expression on the surface of human pDCs was comparable to that of human monocytes, which are known to express Dectin-1. Dectin-1 was upregulated after stimulation with all viral stimuli. Curdlan induced the upregulation of costimulatory markers CD40, CD83, CD80, and CD86, as well as the production of IL-6 (but not IFN-a) and the upregulation of Dectin-1 itself. Finally, pDCs from HIV patients expressed modestly higher baseline levels of Dectin-1 on their cell surface, which remains consistent after curdlan and viral stimulation. We have shown that human pDCs phagocytose and kill Aspergillus spores directly. Dectin-1 expression, regulation, and IL-6 responsiveness of pDCs after curdlan stimulation indicates Dectin-1 is present and functional on human pDCs, suggesting Dectin-1 may be used by human pDCs to recognize the β-glucan component of fungal spores. Finally, the higher baseline Dectin-1 levels on HIV patient pDCs may be explained by low-level chronic immune activation experienced by HIV patients, which has been described to negatively affect pDC functionality.
Poster: #14

The development of protective immunity against Cryptococcus neoformans is controlled by the novel virulence factor Fbp1

J Masso-Silva, V Espinosa, TB Liu, Y Wang, C Xue and A Rivera

Center for Immunity and Inflammation, Rutgers New Jersey Medical School, Newark, NJ

Cryptococcus neoformans is the main etiological agent of cryptococcal meningitis and causes approximately a million deadly infections per year. The vast majority of cryptococcal infections occur in patients with compromised immune function. Although it is well appreciated that adaptive immunity and CD4+ T cells are crucial for defense against cryptococcosis, our understanding of factors that control the development of effective immunity against this fungal infection is incomplete. In previous studies, we identified the F-box protein Fbp1 as a novel determinant of C. neoformans virulence that operates independently of the dominant virulence factors. Deletion of Fbp1 (fbp1¿) in the highly virulent H99 serotype A strain resulted in an hypovirulent phenotype in vivo such that 100% of mice infected with fbp1¿ survived the infection. In this study, we uncovered that the hypovirulence of fbp1¿ yeast is linked to the development of a robust pulmonary inflammatory response and the activation of protective Th1 and Th17 CD4+ T cells in the host. Infection with fbp1¿ induces the rapid influx of CCR2+ monocytes and their differentiation into monocyte-derived dendritic cells (mo-DCs) that orchestrate the activation of fungus-specific CD4+ T cells. Depletion of CCR2+ monocytes and their derivative mo-DCs resulted in impaired activation of a protective inflammatory response and the rapid mortality of mice infected with fbp1¿. Mice lacking B and T cells also developed fungal meningitis and succumbed to infection with fbp1¿ thus demonstrating that the avirulence of this strain is dependent on the activation of protective adaptive immunity in the host. Moreover, we find that the enhanced immunogenicity of fbp1¿ yeast cells can be harnessed to confer protection against a subsequent infection with the virulent H99 parental strain. Altogether our findings suggest that Fbp1 is an important virulence factor in C. neoformans that acts to inhibit the development of protective immunity in the host.
Role of the host Mevalonate Pathway in Mycobacterium tuberculosis infection

Natalie Bruiners
Public Health Research Institute New Jersey Medical School Rutgers, The State University of New Jersey, Newark NJ

There is a growing body of evidence indicating that Mycobacterium tuberculosis (Mtb) utilizes host derived lipids to persist within the host. It is known that Mtb perturbs the host cholesterol biosynthetic pathway, suggesting that mycobacterium interacts with the host mevalonate pathway. This pathway is involved in a number of cellular processes, including protein prenylation and cholesterol synthesis. A critical branch is the processing of farnesyl pyrophosphate (FPP), which is involved in the prenylation via farnesylation or geranylgeranylation, or dedicated to form cholesterol by the conversion of squalene. We therefore aim to investigate the requirement of this pathway for the survival of Mtb in vitro culture by systematically adding mevalonate intermediates and inhibitors of each essential branch of the biosynthetic pathway to evaluate their effects on mycobacterial growth. Interestingly, the addition of geranylgeranyl pyrophosphate (GGPP) inhibited mycobacterial growth. Several proteins undergo posttranslational modification by the addition of geranylgeranyl groups to specific carboxy-terminal CAAX motif by two geranylgeranyl transferases, type I and II. We found that the inhibitory function of GGPP was partially reversed by the addition of the type I inhibitor, this reversal was not observed with the type II inhibitor. The data presented demonstrate that, besides the necessity of cholesterol, processes involving geranylgeranylation may affect infection outcome.
CoCr microparticles induce a robust type 2 innate immune response that is dependent on SYK and BTK signaling

MJ Palma, PK Mishra, KS Beebe, J Benevenia, WC Gause

Center for Immunity and Inflammation, Department of Medicine, Rutgers New Jersey Medical School, Newark, NJ

Cobalt chrome (CoCr) is a major component of orthopaedic implants and a source of metallic wear debris, which may contribute to harmful inflammation and implant failure. Intra-peritoneal inoculation of CoCr induces a robust innate type 2 response with increases in M2 macrophages, eosinophils, and neutrophils and this response is dependent on Spleen tyrosine kinase (SYK) and Bruton’s tyrosine kinase (BTK) signaling, but not TLR, Caspase-1, or Cyclooxygenase signaling. We have also shown that this robust response is independent of T and B cell signaling. To examine this response in a more clinically relevant murine model, bilateral intra-articular knee injections of CoCr microparticles were given to C57BL/6 mice and changes in different innate immune cells were assessed from knee synovial cell isolates. Administration of the SYK inhibitor BAY 61-3606, or the BTK inhibitor Ibrutinib, abrogated innate immune cell recruitment and M2 macrophage polarization which is otherwise observed 2 days after inoculation with CoCr microparticles. The presence of M2 macrophages (CD68+, CD206+, and CD163+) is also observed in human peri-prosthetic tissue samples obtained from patients undergoing revision joint arthroplasty. Taken together, our findings suggest that BTK signaling is required for the type 2 inflammatory response to CoCr microparticles, providing a potential novel target for control of harmful inflammation leading to fibrosis and aseptic loosening.
Poster: #17

Multi-step Commensal-induced Th17 Cell Differentiation in the Mouse Intestine

Teruyuki Sano

Molecular Pathogenesis Program, The Kimmel Center for Biology and Medicine of the Skirball Institute

Th17 cells have significant roles in maintaining homeostasis and regulating host defense against various pathogens in our bodies. Our laboratory initially identified segmented filamentous bacteria (SFB) as a unique commensal that is sufficient for Th17 cell differentiation and promotion of Th17-dependent autoimmune diseases such as a mouse model of spontaneous arthritis. The molecular and cellular requirements of SFB-induced Th17 cell differentiation are still unclear. To understand the whole process of Th17 differentiation in vivo, we developed SFB-specific T cell receptor transgenic (7B8) mice and traced SFB-specific Th17 differentiation and response by transferring fluorescently-labeled 7B8 naïve T cells into SFB-gavaged hosts. Using this approach, we have elucidated the requirements for cytokines and antigen presenting cells (APCs) to understand the process of Th17 cell differentiation. Here, we describe that initial induction and expansion of Th17 cells occurs in mesenteric lymph nodes (MLN), and their subsequent migration to intestine is integrin b7-dependent. Although RORgt expression in Th17 cells is mainly dependent on IL-6 signaling in the MLN, IL-23R signaling also contributes to the RORgt expression in the ileum in the absence of IL-6. CD103+ CD11b+ APCs are not important for induction of SFB-specific Th17 cells in MLN, but play important roles in maintenance of SFB-specific Th17 cells in the ileum. Cytokine-genes expression in Th17 cells in the ileum are further activated by local epithelial production of Serum Amyloid A proteins, which are initiated by SFB-triggered circuit. Taken together, these results indicate that Th17 cell differentiation proceeds in multiple-discrete stages.
Poster: #18

Understanding Borrelia glycosaminoglycan-binding protein one domain at a time: Targeted disruption of heparin binding function

S Schlachter, L Alter, N Parveen
Department of Microbiology, Biochemistry and Molecular Genetics, Rutgers University of Biomedical and Health Science, Newark, NJ

Lyme disease is the most common vector borne illness in the United States with the CDC estimating 300,000 new cases/year. The spirochete bacteria, Borrelia burgdorferi causes Lyme disease and is transmitted to the host by an infected tick bite. Undetected and untreated infection often leads to systemic disease that can affect a patient’s quality of life. The goal of our research is to better understand the contribution of host-microbe interactions in Lyme disease pathogenesis.

B. burgdorferi surface protein, Borrelia glycosaminoglycan binding-protein (Bgp), shows affinity for glycosaminoglycans and exhibits 5’-methylthioadenosine/S-adenosylhomocysteine nucleosidase activity, which likely promotes nutrients salvage in this auxotrophic bacteria. Characterization of the adhesin function of Bgp using 35S-labeled spirochetes showed that the strain lacking Bgp is significantly impaired in binding to endothelial and epithelial cells (p<0.01) in vitro. Furthermore, following targeted mutagenesis of the putative heparin-binding domain, we observed impaired binding of the recombinant mutant protein to purified heparin by ELISA (p<0.01). Binding to other glycosaminoglycans remains unchanged indicating specificity. Our in vitro findings suggest that Bgp likely contributes to vasculature and skin colonization, and may play a role in the associated pathologies in vivo.

C3H mice manifest Lyme disease similarly to humans. Our mouse infection experiments show that the strains lacking Bgp expression are attenuated in infectivity with significant increase in their ID50 compared to wild-type strains. Although this highlights the importance of Bgp for infectivity, the relative contribution of Bgp adhesin and nucleosidase activities during infection has yet to be determined. Our findings suggest that mutants deficient in Bgp heparin-binding are impaired in adherence and may have a role in tissue colonization. Future work with intact strains that lack adhesin but retain nucleosidase function will determine the contribution of Bgp heparin-binding activity during infection. Interestingly, blocking of nucleosidase activity results in B. burgdorferi killing. Characterizing the two domains of Bgp will further our understanding of host-pathogen interactions that support infection and may lead to the identification of novel drugs against this critical protein.
Hematopoietic stem cells (HSCs) are critical for the lifelong production and maintenance of all blood cell types. The molecular mechanisms that guide this process remain poorly understood. The 15kDa proliferating cell nuclear antigen (PCNA) associated factor (Paf) is a potent oncogene that is over-expressed in most cancers. We have previously shown that Paf is essential for HSC and progenitor function and development. Paf deficient mice (Paf-/-) are leukopenic due to reduced number of HSCs and committed progenitors. Paf-/- HSCs failed to maintain quiescence, to self-renew and to support long term hematopoietic reconstitution. To determine the in vivo molecular interactions and pathways by which Paf functions to mediate hematopoiesis, we introduced mutant versions of Paf into the Paf-/- mice. These unique mouse models allowed us to show that Paf-PCNA interactions and Paf ubiquitylation are both essential for hematopoiesis. Furthermore, biochemical analyses of cells from these mice showed that Paf interaction with PCNA was essential for nuclear localization and proper ubiquitylation of Paf. Collectively, therefore, our studies show that Paf function is dependent upon the ability to interact with PCNA and that the ubiquitylation of Paf regulates Paf's function during hematopoiesis. Analyses are ongoing to further delineate the molecular mechanism by which Paf mediates HSC function and development.
Poster: #20

TCR α/β pairing controls recognition of CD1d ligands and directs the development of adipose tissue-specific NKT cells

Joshua A. Vieth, et al.
Child Health Institute of New Jersey Rutgers University, Department of Pediatrics, New Brunswick, NJ

The limited binding footprint, orientation and high affinity of the NKT TCR for its ligand makes this interaction distinct from conventional TCR:MHC interactions. The relative rigidity of the NKT TCR CDR loops is also unique for a TCR. Molecular modeling suggested that a hydrophobic patch created upon TCR α/β pairing might play a role in maintaining the NKT TCR conformation. Disruption of this patch ablated recognition of CD1d, but did not interfere with MHC I or MHC II interactions. Partial ablation, while not compromising CD1d binding, did substantially alter NKT cell development, resulting in to the selective accumulation of adipose tissue-specific NKT cells. Therefore, we have uncovered a key component of the TCR that is essential for the development of a distinct sub-lineage of NKT cells.
Inflammatory bowel disease (IBD) is a condition in which the chronic and recurring inflammatory-immune response is abnormally activated in the absence of a pathogenic threat. IBD is also associated with Colorectal cancer (CRC), with IBD patients at far greater risk to develop CRC. The tumor suppressor gene Smad4 is believed to play essential role in both diseases, since loss of Smad4 can not only lead to hyperactive Wnt signaling pathway which progress CRC formation, but also loses its regulation of anti-inflammatory TGF-β signaling pathway. I found that under the Dextran Sodium Sulfate (DSS) induced colitis model, the presence of Smad4 surprisingly triggers pro-inflammatory response as our results implicate significant weight drop, colon length shortening, and severe crypt damaging and ulceration in control mice compared to mice lacking Smad4. In addition, the transcriptional level of inflammatory cytokines IL-1β and IL-6 are remarkably elevated only in DSS-WT group relative to untreated-WT group. Nevertheless, untreated-Smad4KO group has higher initial of IL-1β and IL-6 mRNA expression than untreated-WT group. Together, these studies indicate that Smad4 plays an anti-inflammatory role under normal conditions, but triggers a pro-inflammatory response during IBD.
Paneth Cell Specific Lysozyme Regulates Intestinal Type 2 Immune Response through Modulating Gut Microflora Composition

Sy Yu, I Balasubramanian, YL Zhao, KL Edelblum, G Yap, N Gao
Department of Biological Sciences; Center for Immunity and Inflammation, Rutgers-New Jersey Medical School, Newark, NJ

Coordinated interactions among gut flora, intestinal epithelial cells, and mucosal professional immune cells maintains a healthy mucosal function. Genetic and environmental disruption of this homeostasis predisposes the individual to inflammatory bowel disease (IBD). Lysozyme is a beta-glycosidase that specifically hydrolyze bacterial peptidoglycan. The intestinal Paneth cells are the major sources of intestinal lysozyme. Abnormalities in Paneth cell lysozyme gene expression or intracellular protein distribution have been reported in IBD patients or animal models deficient for ATG16L, NOD2, and LRRK2. It is not clear whether these lysozyme defects are simply the consequences of IBD pathogenesis, or they may also contribute to disease progression. In this study, we derived two mouse models including Paneth cell specific Lyz1 knockout and ectopic Lyz1 expression driven by Villin1 promoter and explored the role of Lyz1 in modulating gut microbiota composition and contribution to gut mucosal immune homeostasis. Interestingly, we found that the number of goblet cell and tuft cell was increased in Lyz1 knockout mice compared to that in their wild type littermates. We also detected elevated expression of type 2 and 3 cytokines and key transcriptional factors in absence of lysozyme. In situ hybridization and immunofluorescence showed that bacterial invasion events were increased and the thickness of mucin layers compromised in Lyz1 knockout mice. In consistent with these observations, fecal 16rRNA sequencing also indicated several mucolytic bacteria expanded in gut microbiota landscape from Lyz1 knockout mice. Therefore, our results suggest lysozyme may affect intestinal type 2 immune response through modulating gut microflora composition.
Epigenetic signals modulate 1,25(OH)2D3 regulation of innate immune responses in lung epithelial cells

Wei, P. Dhawan, K-Y. Kim, G. Diamond, and S. Christakos
Dept. of Microbiology, Biochemistry and Molecular Genetics, Rutgers New Jersey Medical School, Newark, NJ and Dept. of Oral Biology, University of Florida, Gainsville, FL

A principal defense mechanism protecting the lungs against infection is the production of antimicrobial peptides. We earlier reported that cathelicidin (LL-37) and CD14 (a TLR4 co-receptor that aids antimicrobial peptide production) are induced by 1,25(OH)2D3 in human airway epithelial cells with a resultant increase in antimicrobial activity. Currently very little is known concerning molecular level changes that control the expression of LL-37 and CD14 in lung epithelial cells. We found that butyrate or trichostatin A (histone deacetylase inhibitors; HDACi) significantly enhance 1,25(OH)2D3 induction of LL-37 and CD14 mRNA and transcription in lung epithelial cells (2 – 6 fold). Dominant negative (DN) BRG1 (BRG1 is a component of the SWI/SNF chromatin remodeling complex) inhibits the stimulatory effect of 1,25(OH)2D3, indicating that the SWI/SNF complex is involved in mediating 1,25(OH)2D3 regulation of innate immune responses. Treatment with HDACi relieved the inhibition by BRG1-DN. ChIP/re-ChIP assays showed direct protein-protein interaction between C/EBPα and BRG1 as well as recruitment of BRG1 and C/EBPα to C/EBP sites in the LL-37 and CD14 promoters in response to 1,25(OH)2D3. ChIP/re-ChIP analysis also indicated an increase in acetylated histone 4 in association with BRG-1 in response to 1,25(OH)2D3 at the C/EBP sites. Thus our findings suggest that one mechanism of enhancement of 1,25(OH)2D3 induction of LL-37 and CD14 by HDACi is enhanced cooperation between acetylation and chromatin remodeling (through BRG1) allowing for efficient initiation of transcription. BRG1 can be an activator or a repressor depending on differential cooperation with and recruitment of BRG1 associated factors. We found that protein arginine methyltransferase 5 (PRMT5), a type II methyltransferase, interacts with BRG1 and represses LL-37 and CD14 mRNA and transcription. Our findings indicate the requirement of the C/EBP site for the inhibitory effect of PRMT5. This mechanism of negative regulation may be important for times when induction of LL-37 or CD14 by 1,25(OH)2D3 is not needed or the repression may be involved in the cyclical transcriptional process that requires both activating and repressive epigenetic mechanisms. Our findings define novel mechanisms and key mediators involved in the regulation by 1,25(OH)2D3 of innate immune responses in lung epithelial cells.
Poster: #24

**IL33 rescues CoCr mediated type 2 inflammation after Bruton’s Tyrosine Kinase blockade**

Pankaj K Mishra, Mark Palma, Ariel Millman, Nadim Hallab, Kathleen Beebe, Joseph Benevenia, Thirumala-Devi Kanneganti and William C Gause

Wear debris microparticle release associated with total joint arthroplasty may contribute to harmful inflammation, osteolysis and implant failure. We examined the role of different molecular signaling pathways in microparticle-mediated inflammation. Intra peritoneal inoculation of cobalt chrome (CoCr) microparticle in mice induces a robust innate type 2 response with increases in Th2 cytokines, M2 macrophages, eosinophils and neutrophils when compared to controls given vehicle only. Furthermore, this type 2 innate particle-induced response is independent of MYD88/TRIF and partially dependent on Caspase-1 signaling pathways. Blockade of the SYK signaling pathway with the inhibitor BAY61-3606 or the down stream BTK signaling pathway with Ibrutinib abrogates increase in the alramin IL33, and Th2 cytokines m-RNA. We have further shown that this CoCr mediated type 2 response is independent of B and T cell by employing JH–/– mice and depletion of T cells by anti-CD4 (GK1.5) antibody respectively. Further, studies in CBA/N<sup>xid</sup> mice, which is a genetic BTK loss of function mutant, corroborates the critical role of BTK in CoCr mediated sterile type 2 inflammation. We further demonstrate that Intra-peritoneal administration of recombinant IL-33 rescues the CoCr mediated type 2 response in the context of BTK blockade with iibrutinib. These studies suggest that the type 2 immune response caused by solid particles is mediated through BTK signaling pathways and is dependent on IL-33. These findings provide potential targets for controlling the wear debris-mediated type 2 sterile inflammation that can contribute to aseptic loosening and implant failure.
Identifying blood signature for predicting tuberculosis disease progression

Samantha Leong¹, Tyler Faits², W. Evan Johnson², Rodrigo Ribeiro Rodrigues³, Edward C. Jones-López⁴, David Alland¹, Reynaldo Dietze³, Jerrold J. Ellner⁴, and Padmini Salgame¹

Affiliation/Institution:
1 Department of Medicine, Center for Emerging Pathogens, Rutgers New Jersey Medical School, Newark, NJ, USA
2 Division of Computational Biomedicine, Boston University School of Medicine, Boston, MA, USA
3 Universidade Federal do Espírito Santo, Núcleo Doenças Infecciosas, Vitória, ES, Brazil
4 Section of Infectious Diseases, Boston Medical Center, Boston, MA USA

Clinical management of individuals latently infected with *Mycobacterium tuberculosis* (Mtb), an estimated one-third of the world’s population, remains a challenge due to lack of diagnostics that can predict which individuals will progress to active tuberculosis (TB) disease or not. The overall goal of this study was to assess the ability of a transcriptomic signature of baseline peripheral blood samples from Mtb-infected subjects to classify individuals as eventual progressors or non-progressors. From March 2008 to June 2012, we screened 293 TB patients to enroll 124 index cases and their 731 contacts in a household contact study in Brazil. Baseline peripheral blood mononuclear cell (PBMC) samples were isolated from subjects at the time of enrollment, and the contacts were passively followed to determine whether they progressed to TB disease. In total, 32 contacts were later identified to have progressed to TB disease. This training set of subjects consisted of 19 progressors and 9 non-progressors, and 9 index cases were also studied as controls. RNA was extracted from PBMCs and sequenced using Illumina HiSeq 2500. DEseq was used to identify a list of differentially expressed genes between non-progressors and progressors, from which candidate predictor genes were selected using the glmnet elastic net method. Nineteen candidate genes were identified, which were found to perform robustly ($r = 0.985$) upon bootstrapping to evaluate classification performance. Additional studies in a test set and independent validation set are ongoing.
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